

Research article

## Detection of *Escherichia coli* O157:H7 in Food

Zahraa A. Jabur<sup>1\*</sup>, Saad S. Fakhry<sup>1</sup>, Manal A.Hassan<sup>1</sup>, Bashar qussay kadhem<sup>1</sup>

### ABSTRACT

The present study aims to estimate the presence of *Escherichia coli* O157:H7 as a contamination indicator in beef meat, dairy products and different types of ready food in local markets at Baghdad city. Sixty one samples of different food samples were tested. One isolate was detected, *E. coli* O157:H7 on differential and selective media MacConkey agar, sorbitol MacConkey agar, chromogenic media and routine biochemical tests were used to diagnose and identify the bacteria of other members of enterobacteriaceae. Seven isolates of *E. coli* were confirmed on Hi Crome EC 0157:H7 selective agar as *E. coli* O157:H7, then results showed one isolate sorbitol negative carrying Shiga-like toxins (SLT)2 was detected by PCR as *E. coli* O157:H7, during experiments another isolate was sorbitol positive also carried SLT2. The duplex PCR procedure did not detect the *SLT1* sequence in any of the contaminated samples, but found *SLT2* genes in one of them. The current study supported that the presence of *E. coli* O157:H7 is low in food.

**Keywords:** *Escherichia coli* O157:H7, PCR, Chromogenic media, Oligonucleotide primer, High toxin-producing

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## INTRODUCTION

High toxin-producing *Escherichia coli* (STEC), also known as verocytotoxin-producing *E. coli*, are important food borne pathogens responsible for outbreaks of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). STEC that cause HC and HUS are also referred to as enterohemorrhagic *E. coli* [1]. Enterohemorrhagic *Escherichia coli* O157:H7 is an important foodborne pathogen a causative agent of hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS). Large outbreaks of EHEC infection were reported throughout the world. *Escherichia coli* O157:H7 is the most commonly recognized STEC in the United States, however, many other STEC serogroups including O26, O103, O111, and O145, have been associated with outbreaks and sporadic cases of HC and HUS worldwide [2]. One of the largest *E. coli* O157:H7 (one of the serotypes of EHEC) outbreaks associated with food consumption occurred in Sakai City, Japan in 1996 [3].

*E. coli* O157 and *E. coli* O157:H7 are present in the intestines of cattle as a component of the native microbiota and they can contaminate both the meat and the slaughterhouse environment. Due to the result, contamination of both carcasses and the environment by *E. coli* O157 and *E. coli* O157:H7 from the intestinal contents of cattle during slaughter is one of the most significant risk factors intrans mission to humans [4,5]. The pathogenicity of *E. coli* O157 and *E. coli* O157:H7, including STEC, are associated with several virulence factors. The main factor contributing to their pathogenicity is their capacity to produce two potent phage encoded cytotoxins called Shiga-toxins (namely, *Stx1* and *Stx2*) [6]. The production of toxins, another virulence-associated factor expressed by STEC is a protein called Intimin, which is encoded by the *EAE* gene and responsible for the intimate attachment of STEC to the intestinal epithelial cells [7].



\*Correspondence: Jabur.ZA.zahraa.abbas26@yahoo.com

Ministry of Higher Education and Scientific Research, Water and Environment Directorate, Food Contamination Research Center, Baghdad – Iraq.

Full list of author information is available at the end of the article.

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It has been demonstrated that certain *E. coli* isolates produce toxin, which was initially called Vero toxin because of its distinct effect on Vero cells<sup>1</sup>. This family of toxins was subsequently also called Shiga-like toxins (*SLT*), and more recently Shiga toxins (*Stx*), because of the close relation to the *Stx* of *Shigella dysenteriae* type 1. It has been demonstrated that certain *E. coli* isolates produced a toxin, which was initially called Vero toxin because of its distinct effect on Vero cells<sup>1</sup> [8,9].

Were many researches proved internationally, decreasing the ratio of *E. coli* O157H7 isolation in third world and Middle East for eg. Iraq, also a distinguish spreading at the northern part of earth, thus may belong to demonstrated social habits in preparing food. Therefore it cannot be considered an epidemic [10]. But in recent years there were many studies in Iraq about infection with *E. coli* O157H7 which caused diarrhea for children after ingestion contaminated food and in a few cases can cause death for them [11].

At this present study one of aims was isolating *E. coli* O157:H7 from food samples by conventional culture method and confirmation by a specific multiplex-PCR assay to determine *stx1* and *stx2* genes presence.

## MATERIALS and METHODS

### Food sampling

A total of different of 61 varied food sample of meat, minced meat, meat burgers, milk, dairy products, cooked food and ready foods in addition to several types of salads, were collected randomly from local markets at Baghdad city, after transfer them to food contamination center, samples were analyzed and identified according to the type of sample.

### Isolation of *E. coli*

25 gm of food samples was taken and homogenized with 225 mL of buffered peptone water 0.1% in a stomacher for one min, then the homogenate was diluted serially and 0.1 ml of each dilution were plated three media, MacConkey agar and Sorbitol Macconkey agar (SMAC) supplemented with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) were used to detect *E. coli* and EHEC colonies. McA and SMAC agar after culturing with bacterial cultures, incubated for 24 h at 37°C. Seven lactose fermenting colonies from MacConkey agar and non-sorbitol fermenting (colourless) colonies from SMAC agar were transferred individually to fresh nutrient broth and incubated at 37°C for 18 h, then streaked on CHROM agar O157 and on plates containing eosinmethylene blue agar (EMB) then incubated at 37°C for 24 h. These isolates, with typical *E. coli* metallic sheen on EMB, were characterized by biochemical tests, including conventional Gram staining, indole production, methyl red, vogesproskauer, citrate utilization and urease and hydrogen sulphide was used to confirm the *E. coli* species.

### DNA extraction

DNA of identified cultures of *E. coli* O157:H7 was extracted from whole cell suspensions according to modified previous method [12]. Harvesting cultures of *E. coli* O157:H7 were incubated at 37 °C for overnight. Then 2 ml of culture was centrifuged at 14,000 g for 10 min. pellet was resuspended

in 500 ml of TE buffer (1M Tris PH8, 0.5M EDTA PH8, dH<sub>2</sub>O), then 50 µl SDS (10%) were added with 25 µl of proteinase K (20 mg/ml), later mixed by inverting the tube several times and incubate for 30 min at 55 °C, and 75 µl of a mixture of phenol/chloroform in ratio (1:1) were added and mix gently by inverting the tube several time until it a homogenous milky solution, the mixture of sample and additives are centrifuged for 10 min at 14000 g. Carefully transferred 500 µl of the upper aqueous phase into a new tube later, a volume of 500 µl from phenol/chloroform in ratio (1:1) were added, invert the tube gently to homogenize the mixture and spin down for 10 min at 14000 g and then, 500 µl of upper phase was put into a new tube and 50 µl of sodium acetate was added and mix gently. Then, 330 µl of iso-propanol was added and mix gently by inverting the tube to precipitate DNA then washing it several time by 70% ethanol. After that, the pellet was dried by keeping tube open for 10 min and resuspended in 50 µl of distilled water, finally DNA integrity was assessed by running in 0.7 µl agarose gel then visualized by gel-documentation (Bio-rad).

### Primers

The presence of virulence associated genes i.e. shiga-like toxin (*SLT1* and *SLT2*), heat stable toxin of *E. coli* were determined by PCR. Primers used for associated genes are depicted in **Table 1**.

**Table 1.** Oligonucleotide primer sets used in PCR.

Primer sequence	Annealing Temperature (c)	Product size (bp)	Ref.
SLT1: F-GAAGAGTCCGTGGGATTACG R-AGCGATGCAGCTATTAATAA	55	130	[13]
SLT2: F-TTAACCACACCCACGGCAGT R-GCTCTGGATGCATCTCTGGT	55	346	[13]

### PCR gene reaction

PCR reaction to amplify of *SLT1* and *SLT2* genes was prepared by mixing 25µl of reaction in 0.2 ml PCR tube by adding 2 µl of DNA which used as a template and 20 Pico molar solution of each primer, in addition to 1X Taq amplification buffer, also 1mM MgCl<sub>2</sub> were added and 200 µM of each nucleotides also 1.25 units of Tag DNA polymerase were used for each reaction. The mixture containing PCR tubes were quickly spun at 10000 g for few seconds and placed in gradient thermal cycler, cycling condition for amplification included 94°C for 3 min, for annealing 1 min and 72 °C for 1min followed by 72 °C for 10 min. positive amplification was visualized by electrophoresis of the product in 1.7% agarose gel stained with ethidium bromide in a submarine horizontal electrophoresis apparatus.

## RESULTS AND DISCUSSION

Results showed that from 61 food samples, 21 (34.4%) were *E. coli* which isolated on McA and SMCA agar. These isolates are confirmed as *E. coli* by biochemical tests. From 21 positive results on MacConkey agar and SMCA as *E.*

*coli*, seven isolates were confirmed on HiCrome EC O157:H7 selective agar as *E. coli* O157:H7, morphologically, formed dark purple to magenta colored moiety on HiCrome medium (Table 2).

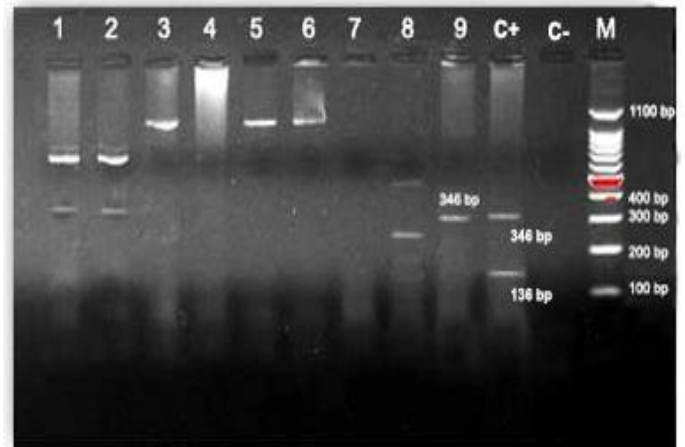
**Table 2.** Percentage of *E. coli* O157:H7 in food sample

Food sample	Sample (-)	Sample (+)	Percentage (%)
Meat and product	19	1	5.2%
Yoghurt and product	27	1	3.7%
Rice	6	0	...
Salads	7	0	...

One isolates sorbitol negative carrying *SLT2* was detected by PCR to identify as *E. coli* O157:H7, and another isolate which sorbitol positive also carried *SLT2*. Furthermore, the results showed that one isolate of *E. coli* O157:H7 was detected from 61 food samples. Therefore, the fact that the occurrence of *E. coli* O157:H7 was very low, it must be taken into consideration that the cultural methods used in this study may contribute to the low isolation rate. The exact contamination rate may be at least two times higher than stated here due to the low isolation rate of culture methods compared to other immunological and genetical methods [14]. Similar study carried out in Iraq, from 100 samples of meat only two isolates and from 98 dairy product samples were detected as *E. coli* O157:H7 [15]. In Iran, another study proved that, from 130 bulk tanks of milk just one isolated was *E. coli* O157:H7 [16]. While, in another study, from 125 sample of soft cheese prepared from raw milk, found 5 isolates of *E. coli* O157:H7 [17]. Out of 50 ground beef samples 7 strains of *E. coli* O157:H7 were detected, while not isolated the organism from chicken drumsticks in Turkey [18]. PCR assay was used for confirming the non-sorbitol fermenting colonies as *E. coli* O157:H7. Several researchers proved that multiplex PCR as a reliable identification method for *E. coli* O157:H7 [19]. The particular genes was chosen for the reason that the simultaneous targeting of *SLT1* and *SLT2* sequences was important in detection of foods contaminated with a virulent STEC.

In the multiplex-PCR assay the presence of the main virulence genes (*stx1* and *stx2*), which have been widely used by other researchers [20,21]. According to our results, *stx2* was detected in isolated *E. coli* O157:H7, but *stx1* was not detected. It has been reported that the *stx2* gene was more common than the *stx1* (Fig. 1). This agreed with studies performed in the USA, Japan and European countries [20, 22,23].

The duplex PCR procedure did not detect the *SLT1* sequence in any of the contaminated samples, but found *SLT2* genes in one of them. The possible explanations are that these were true findings, food samples contained bacteria producing *SLT2*. The primer pair amplified more than one PCR product in three isolates, which suggest the presence of different allelic forms of the *SLT* gene. The result concluded that meat and dairy product samples analyzed were harbored virulent genes for Shiga like toxins. It pointed out that samples contaminated with *E. coli* strains in such



**Fig 1.** Agarose gel electrophoresis of PCR products to detect *SLT2*. M (DNA marker), C- (negative control), C+ (Positive control)

level could produce potential hazard for consumers. Next to that, further exploration of other virulent strains like enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAggEC) are needed to know the particular situation of *E. coli* contaminated food at local market. It can be concluded from current study that the level of presence of *E. coli* O157:H7 in different food samples. The duplex PCR procedure did not detect the *SLT1* sequence in any of the contaminated samples, but *SLT2* gene was found in one of them. This study supported that the presence of *E. coli* O157:H7 in food samples is low.

**Conflict of interest**

The authors declare that they have no conflict of interests.

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*Author affiliation*

1. *Food Contamination Research Center, Ministry of Higher Education and Scientific Research, Water and Environment Directorate, Baghdad, Iraq*

